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14. ABSTRACT

This proposal aims to test the hypothesis that integrating observations derived from mouse model systems with observations from human prostate cancers will define relevant and consistent molecular alterations critical to the development and progression of prostate carcinoma. The research accomplished to date has: 1) assembled the requisite mouse models to enable the generation of tumor gene expression data; 2) produced a second-generation mouse prostate microarray that will allow for deeper profiling of mouse prostate gene expression; 3) identified a specific gene (osteopontin) commonly associated with multiple mouse prostate cancer models; 4) developed the methods/techniques that will enable precise dissection of mouse prostate epithelium; 5) expanded the Prostate Expression Database to archive microarray data; 6) determined strain-specific gene expression differences in the mouse prostate that could contribute to phenotypic differences on prostate cancer development and progression; and 7) identified developmental pathways altered in the Pten-/- prostate cancer model that could contribute to the process of carcinogenesis.

15. SUBJECT TERMS
Database, mouse model, gene expression, microarray, transcript, proteomics

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INTRODUCTION

This proposal aims to test the hypothesis that integrating observations derived from mouse model systems with observations from human prostate cancers will define relevant and consistent molecular alterations critical to the development and progression of prostate carcinoma. Ultimately, these studies will identify those mouse models most accurately reflecting *in vivo* human prostate cancer, and prioritize those genes in human prostate cancer that are most relevant for therapeutic intervention.

The aims of the proposal are: (1) To determine transcript expression profiles of neoplastic lesions from mouse models of prostate carcinoma. (2) To determine the profile of serum proteins that reproducibly associate with distinct mouse models and stages of prostate carcinoma. (3) To stratify mouse models of prostate carcinoma through comparative analyses with clinical human prostate carcinomas. (4) To extend the utility of the Prostate Expression Database to facilitate comparative studies of mouse and human prostate carcinoma.

Disease relevance: Model systems represent critical resources supporting essentially all facets of research involving prostate cancer including studies focused on disease etiology, disease progression, diagnostics, dietary factors, immune modulation, imaging, and pharmacologic intervention. Mouse models offer opportunities for testing hypotheses that would be difficult or impossible to evaluate in humans. Similarly, databases of sequence, gene expression, and disease model information also greatly facilitate scientific work in an extremely cost- and time-effective manner. There is a crucial need to develop interactive resources that generate, compile, and distribute relevant data correlating mouse prostate cancer models directly with phenotypes and genotypes of human prostate carcinoma so as to interpret experimental findings in the appropriate context, determine disease relevance, and prioritize model systems for appropriate pre-clinical studies. This proposal aims to address these needs.

BODY

The following summarizes the technical objectives for the proposal and the work accomplished during the 12-month interval between the start of the project (12/06/04) and the preparation of this report (12/05/05).

D.1.Technical objective 1: To determine transcript expression profiles of neoplastic lesions from mouse models of prostate carcinogenesis (Months 1-24).

Objective 1a. Microdissect specific epithelial populations of cells at discrete stages of prostate carcinogenesis: PIN, invasive carcinoma, metastasis.

Task 1: Breed and microdissect PIN models (months 1-12). We have obtained and bred mouse prostate cancer models of the following genotypes: Nkx3.1-/-, and acquired prostates with PIN lesions from the PB-RXR-/- mouse. The FGF8 mouse is no longer available (Dr. Roy-Burman, personal communication). Thus we will use the expression profile from the Akt-/- mouse developed by Dr. William Sellers as an alternative. We have acquired gene expression data from prostates of these mice which develop PIN but not invasive cancer. We have now microdissected PIN lesions from the Nkx3.1-/- prostates.

Task 2: Breed and microdissect PIN and progression models (months 12-24. We have acquired, bred, and harvested prostates at the PIN and invasive cancer stages from the PB-PTEN-/- and TRAMP models. Microdissection of these lesions is in progress.

Objective 1b. Measure transcript levels in specific epithelial populations of cells at discrete stages of prostate carcinogenesis: PIN, invasive carcinoma, metastasis.

*Task 3: Construct microarrays (months 1-6).*We have constructed a 2nd generation mouse prostate microarray that now comprises ~16,000 cDNAs representing ~12,000 unique genes. This array has been quality checked for sequence accuracy and reproducibility. Preliminary experiments using microdissected cancerous mouse prostate epithelium demonstrated high quality hybridization results.

Task 4: Amplify RNA from microdissected mouse prostate tissue (months 6-24). We have microdissected normal and neoplastic mouse prostate epithelium from the PB-PTEN model, amplified the RNA, and verified the quality of the aRNA using the Agilent bioanalyzer.

Task 5: Hybridize mouse prostate cDNA probes to microarrays (months 6-24). We have hybridized amplified RNA from cancerous and benign mouse prostate epithelium in a comparative manner. The preliminary analysis of these PTEN-/- experiments identified ~400 genes differentially expressed between benign and neoplastic cells, with several lobe-specific differences. We are finalizing a study evaluating strain-specific differences on mouse prostate gene expression that may identify host differences accounting for different cancer penetrance rates (Fig 1). This manuscript is in preparation for submission. We have also identified pathways of normal mouse prostate development that are reactivated in the setting of neoplasia. We are now confirming the altered expression of these genes by qRT-PCR. The mechanistic analysis of one gene we found to be altered in multiple prostate cancer models, osteopontin, has been accepted for publication in *Cancer Research*.

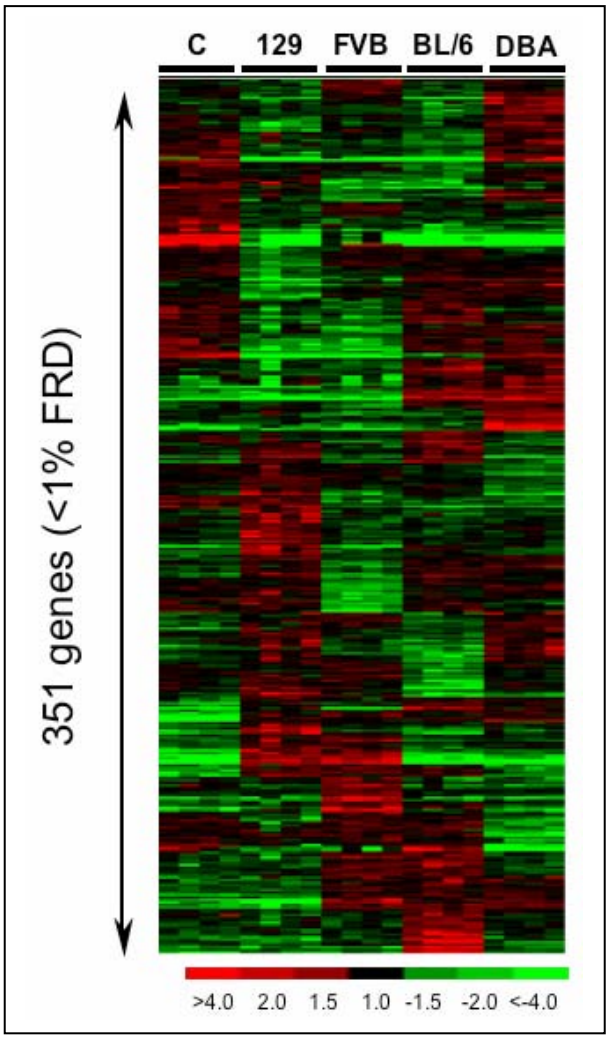

Fig 1. Comparisons of prostate gene expression across 5 inbred mouse strains identified 351 genes with strain-dependent differential expression. Several genes (e.g. clusterin) have known roles in prostate carcino-

<u>Objective 1c.</u> Identify expression alterations that are common to specific stages of neoplastic growth.

Task 6: Format and QC microarray data (months 18-24). In progress.

Task 7: Statistical analyses of microarray data (months 18-24). In progress.

<u>D.3. Technical objective 2:</u> Stratify mouse models of prostate carcinoma through comparative analyses with clinical human prostate carcinomas (months 18-34).

All objectives in this aim are for months 18-34.

D.4. Technical objective 3: To extend the utility of the Prostate Expression Database (PEDB) for comparative gene expression studies of mouse and human prostate carcinoma. (months 10-34)

Objective 3a. Construct an interactive repository for microarray information that integrates multi-dimensional data (tissue type, quantitative and temporal gene expression measurements) for independent analyses.

Task 14: Determine a server configuration for a microarray database server (month 10). We have selected a server configuration using the opensource Bioconductor platform written in the 'R' language. We have begun populating the database with microarray data generated from the mouse model experiments described above.

Task 15: Install and update server configuration for security and accessiblity (month 10). Completed.

Task 16: Reconfigure the PEDB website to use PHP for faster data access and improved interactivity. (months 10-18). Completed.

The remaining objectives for this aim are planned for months 12-34.

Objective 4: Final Report: Complete data analyses, compile accomplishments and reportable outcomes and write final project report (Months 35-36).
This objective is planned for completion in months 35-56.

KEY RESEARCH ACCOMPLISHMENTS

- Acquired the mouse prostate cancer models with specific genetic alterations leading to PIN or invasive cancer (Nkx3.1, RXRalpha, PTEN-/-, TRAMP) and gene expression data from the mouse prostate Akt model.
- Completed the construction and q/c of a second generation mouse prostate specific microarray that nearly doubles the gene expression representation relative to version 1.
- Completed the wet-lab experiments evaluating strain-specific differences in mouse prostate gene expression that could influence the development and/or progression of genetically-engineered prostate cancer. A manuscript describing these results is in preparation.
- Completed microdissection, amplification, and microarray analysis of benign and neoplastic epithelium from the PTEN-/- mouse prostate cancer model system. This analysis has identified several developmental pathways that appear to be re-activated in prostate adenocarcinoma (e.g. Wnt pathway). Analysis of PIN lesions from this model is in progress. Analysis of stromal changes from this model is also in progress.

REPORTABLE OUTCOMES

Ani C. Khodavirdi, Zhigang Song, Shangxin Yang, Hong Wu, Colin Pritchard, Peter Nelson, and Pradip Roy-Burman. (In Press). *Increased Expression of Osteopontin Contributes to the Progression of Prostate Cancer. Cancer Research*.

Colin Pritchard, Madhuchhanda Bhattacharjee, Sarah Hawley, Ruth Dumpit, Robert Sikes, and Peter S. Nelson. *Gene Expression Patterns of Androgen-Regulated Prostate Development: Implications for Prostate Carcinogenesis* (*submitted*).

CONCLUSIONS

The research accomplished to date has: 1) assembled the requisite mouse models to enable the generation of tumor gene expression data; 2) produced a second-generation mouse prostate microarray that will allow for deeper profiling of mouse prostate gene expression; 3) identified a specific gene (osteopontin) commonly associated with multiple mouse prostate cancer models; 4) developed the methods/techniques that will enable precise dissection of mouse prostate epithelium; 5) expanded the Prostate Expression Database to archive microarray data; 6) determined strain-specific gene expression differences in the mouse prostate that could contribute to phenotypic differences on prostate cancer development and progression; and 7) identified developmental pathways altered in the Pten-/- prostate cancer model that could contribute to the process of carcinogenesis.

REFERENCES

None.

APPENDICES

None.